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Phytochemical screening and GC – MS analysis of Methanolic extract of *Ficus racemosa*

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Abstract

Ficus racemosa Linn., belonging to the family Moraceae, is commonly referred to as the cluster fig tree or Gular. It is a medium-sized tree widely distributed throughout India, growing either in the wild or cultivated for its fruits, which are consumed by villagers. In traditional Indian medical systems such as Ayurveda and Unani, *Ficus racemosa* has been extensively utilized for treating various ailments and conditions including skeletal diseases, diabetes, inflammation, hyperlipidemia, hemorrhoids, respiratory issues, liver dysfunction, cough suppression, hepatoprotection, antimicrobial properties, and gastrointestinal disorders. The present study aims to provide information concerning the phytochemical composition and GC-MS analysis of *Ficus racemosa*. The plant has been subjected to phytochemical analysis, revealing numerous bioactive constituents across different parts of the plant. Given the recent accumulation of research findings, the significance of understanding this plant, its traditional uses, phytoconstituents, and biological effects cannot be overstated.

Keywords: *Ficus racemosa*, phytochemicals, methanolic extract, GC-MS analysis

Introduction

For centuries, medicinal plants have served as vital sources of medicine through diverse cultures. Their extensive use in healthcare preparations, as documented in ancient texts like the Vedas and the Bible, underscores the presence of natural products endowed with medicinal properties [1]. In many developing nations, traditional medicine and the utilization of medicinal plants form integral components of healthcare practices aimed at maintaining well-being [2]. Moreover, industrialized societies increasingly rely on medicinal plants, extracting and developing numerous drugs and therapeutic compounds from both traditional and rural remedies [3]. According to the World Health Organization, approximately 80% of the global population relies on botanical medicine for their primary healthcare needs [4].

Ficus racemosa Linn. (Moraceae) is an evergreen tree characterized by its moderate to large size, spreading branches, and deciduous nature, typically reaching heights of 15-18 meters [5]. Belonging to the expansive *Ficus* genus, which comprises over 700 species distributed across Asia, Africa, America, and Australia, *Ficus racemosa* stands out due to its distinctive reproductive system involving synconia fig and specialized pollinator wasps [6, 7]. Commonly referred to as 'gular,' all parts of the *Ficus racemosa* plant hold medicinal significance in Ayurveda, being extensively utilized in the treatment of various ailments including biliary disorders, jaundice, dysentery, diabetes, diarrhea, and inflammatory conditions [8-10].



Fig 1: *Ficus racemosa* plant with unripe fruits

Plant Profile**Species:** *Ficus racemosa***Synonyms***Covellia glomerata* (Roxb.) Miq.*Ficus glomerata* Roxb.*Ficus vesca* F. Muell. ex Miq.*Ficus semicordata* F.M. Bailey**Vernacular names**

English: Cluster fig, Country fig, Redwood fig

Chinese: Ju Guo Rong

Burmese: Jagyadumbar

Hindi: Gular

Urdu: Dimitri

Sanskrit: Udumbara

Kannada & Tamil: Atti

Bengali: Dumur

Distribution

Ficus racemosa found in wet areas, beside streams, on the sides of ravines, and occasionally almost gregariously on rocky slopes over the majority of India. It is also found in Burma, China, Indonesia, Malaysia, and Australia. It is often cultivated around villages in India for its edible fruits [11-15].

Plant Morphology

Ficus racemosa is a deciduous tree capable of reaching a height of 30 meters. It features a buttressed bole with bark measuring 8 to 10 millimeters thick, possessing a surface that is smooth, coarsely flaky, and fibrous, with a creamy pink blaze, and milky latex. Young shoots and twigs are initially finely white and hairy, but they soon become glabrous. Branchlets are 1.5 to 3 millimeters thick and pubescent. Additional components of the plant include.

Leaves

The leaves grow in large clusters at old nodes of the main trunk, are dark green, 6-10 cm long, and glabrous. The receptacles are small, subglobose, or piriform.

Bark: The bark presents a velvety, greyish, or reddish-grey surface (0.7-1.9 cm). Both the inside and outside are light browns, possessing a mucilaginous taste and lacking distinctive odor.

Latex: The mucus-like substance referred to as latex is secreted from the outwardly sliced portion of the bark.

Flowers: A portion of the Ficus fig bears thousands of blossoms in its graceful, attractive flower. Moreover, it exhibits positive biological effects in ophthalmology, antiulcer treatments, and heart diseases.

Fruits: Enormous clusters of pyriform fruits, measuring 3-6 cm in diameter and 1.5 to 2-inch long, are produced by the main trunk or major branches in a rosette-like fashion. Initially green and resembling figs, these fruits mature to orange, dull reddish, or dark crimson.

Seeds: Numerous tiny granular seeds are present. The exterior layer of the bark consists of uniformly hard, easily detachable transparent flakes ranging in color from grey to rusty brown.

Root: The roots of the fig tree exhibit a lengthy, asymmetrical shape and size, with a dark hue, strong odor, and somewhat bitter flavor. They possess both internal and external uses, including wound healing, bone fracture treatment, and anti-inflammatory activity [16-27].

Phytochemistry: *Ficus racemosa* Linn species contain flavonoid glycosides, alkaloids, phenolic acids, steroids, saponins, coumarins, tannins, and triterpenoids, including oleanolic acid, ursolic acid, hydroxy ursolic acid, protocatechuic acid, and malonic acid. Flavonoids, vitamin C, and phenolic chemicals are among the non-enzymatic components. The plant also contains phytochemicals related to flavonoids with isoprenoid substituents and stilbenes. Enzymatic components include ascorbate oxidase, ascorbate peroxidase, catalase, and peroxidase, along with phenolic chemicals such as gallic acid and ellagic acid [28-30].

Table 1: Some important phytoconstituents of *Ficus racemosa*

S. No.	Plant parts	Phytoconstituents
1.	Leaves	Triterpenoids (Lanosterol), alkaloids, and sterols are some examples of plant compounds. The leaves contain a novel tetra cyclic triterpene fluoroacetate, with characteristics including racemosa acid, 13H, 14-H, 17-H, 20-H-lanosta-8, and 22 Diene-3-acetate [31].
2.	Fruits	Hentriacontane, flavanol, flavonoids acetate, glucose, tiglic acid, and ester softer ax a sterol, lupeol acetate, Friedel in, higher hydrocarbons, and another Phyto sterol are among the Compounds that make up flavanol [32].
3.	Stem barks	Saponin gluanol acetate, lau anol acetate, lupeol, cerylbehenate, lupeol acetate, -amyr in acetate, leucoanthocyanidin, and leucoanthocyanins. lupeol, sitosterol, and stigmasterol Were extract ed. from the stem [33].
4.	Trunk barks	Upenol, β -sistosteroland stigma sterol.
5.	Root	Cycloartenol, phorbol, and it's he xacosanoate, taraxerone, tinytoxin; barkphorbol and its hexacosanate, ingenol and its triacetate, taraxerone [34].
6.	Latex	A-Amyrin, Sitosterol, Cycloartenol, Cyclo euphordenol, 4-Deoxyphorbol, and its Esters, Support, Euphorbinol, Isoeuphorbol, Palmitic Acid, Taraxerol, Tiny Toxin, Trimethyl ellagic Acid [35].

Medicinal Importance

Ficus racemosa, extensively cultivated across India, is reputed to harbor a diverse array of therapeutic properties. Historically, various parts of the plant have been employed as fodder. Within the traditional Indian medical system, every component of this plant, comprising the leaves, fruits, bark, latex, and sap of the root, is deemed medicinally significant traditional uses. The roots find application in treating dysentery, pectoral complaints, diabetes, and are applied in cases of mumps and other inflammatory glandular

enlargements. Highly effective in threatened abortion, the bark is also recommended for urological disorders, leucorrhoea, diabetes, hiccups, leprosy, dysentery, asthma, and piles. Leaves serve as excellent washing agents for wounds and ulcers. The infusion of bark and leaves is utilized as a mouthwash for spongy gums and internally for dysentery and menorrhagia. Flowers exhibit positive biological effects in ophthalmology, antiulcer treatments and heart diseases. Tender fruits possess astringent, stomachic, refrigerant properties, alleviating dry cough, loss of voice, kidney

diseases, and acting as a styptic tonic for the spleen. They are useful in the treatment of blood disorders, burning sensations, fatigue, intestinal worms, and as carminatives. It is also believed that bathing with a combination of fruit and bark cures leprosy. Latex is considered aphrodisiac and is administered for diarrhea, diabetes, and boils, reducing edema in adenitis, orchitis, and traumatic swelling. Seeds are used to treat skeletal disorders, diabetes, and angina [36-43].

Materials and Methods

Collection of plant material

Fresh fruits of *Ficus racemosa* were collected from Adhodibavi, near chodeswari Devi temples, Sibyala, Rayachoti, Annamaya Dist. The flowers were identified and authenticated by Prof. N. Savithamma, Professor, Department of Botany, S V University, Tirupati with the voucher number 360.

Preparation of Methanolic Extract

Fresh fruits of *Ficus racemosa* were collected, washed with alcohol and subjected to maceration using methanol for 5-7 days. Further it was extracted using filter paper and subjected to phytochemical screening followed by GC-MS analysis.

Phytochemical analysis

Preliminary Screening of Secondary Metabolite

The crude oil obtained from *Ficus racemosa* flowers was subjected to phytochemical screening based on the procedures outlined by Harborne, Trease and Evans, Harborne, and Soni and Sosa [44-51].

Test for fatty acid & Oils

To 1ml of extract, a few drops of Sudan III solution were added. A shining orange colour obtained showed the presence of fixed oil and fat.

- Filter paper test:** A small quantity of different extracts was separately pressed between two filter papers. Appearance of an oil stain on the paper indicates the presence of fixed oil.

Test for Carbohydrates

- Fehling's test:** 5 ml of Fehling's solution was added to 0.5 mg of bark extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.
- Iodine test:** 3mL extract solution + few drops of iodine solution A blue colour, which disappears on boiling and reappears on cooling
- Molish's test:** 2mL filtrate + 2 drops of alcoholic α -naphthol + 1mL conc. H₂SO₄ (along the sides of test tube) A violet ring

Tests for Tannins

The test solution of the extract was dissolved in minimum amount of water separately, filtered and filtrates were then subjected to the following tests:

- Lead acetate test:** Few drops of aqueous basic lead acetate solution were added to the filtrate. Reddish brown bulky precipitate indicates the presence of tannins.
- 10% NaOH test:** 0.4mL plant extract + 4mL 10% NaOH + shaken well Formation of emulsion (Hydrolysable tannins)

Test for Alkaloids

The test solution of the extracts was dissolved in chloroform and the solution was extracted with Dil. HCl or Dil. H₂SO₄ and acid layer was taken and tested for presence of alkaloids.

- Wagner's test:** Wagner's reagent (Iodine in potassium iodide) was added to the acidic solution. The formation of reddish brown precipitate indicates the presence of alkaloids.
- Dragendorff's reagent test:** 2 ml of Dragendorff's reagent and 2 ml of Dil. HCl were added to the test solution. An orange-red coloured precipitate indicates the presence of alkaloids.
- Mayer's/ Bertrand's/ Valser's test :** Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube) A creamy white/yellow precipitate

Test for saponins

- Foam test:** 0.5gm plant extract + 2mL water (vigorously shaken) Persistent foam for 10 min.

Test for proteins

- Millon's test:** 2mL filtrate + few drops of Millon's reagent A white precipitate

Test for amino acids

- Ninhydrin test:** 2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone) A purple coloured sol. {Amino acids}

Test for steroids and terpenoids

- Salkowski's test:** Filtrate + few drops of conc. H₂SO₄ (Shaken well and allowed to stand) Golden yellow layer (at the bottom)

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry (GC-MS) analysis is a combined analytical technique used to identify and quantify compounds within a sample. This method involves separating and detecting individual components of a complex mixture, making it a powerful tool for chemical analysis. During GC-MS analysis, a sample is vaporized and introduced into a gas chromatograph, where it is separated into its individual components based on their physical and chemical properties. The separated compounds then enter a mass spectrometer, where they are ionized and their mass-to-charge ratios are measured. The resulting mass spectrum provides information about the identity and abundance of the compounds present in the sample. GC-MS analysis is widely used in various fields, including Pharmacy, chemistry, biochemistry, environmental science, and forensic science for the characterization of organic compounds in different samples.

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μ m df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260 °C during the chromatographic run. The 1 μ L of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240

°C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Identification of Compounds

The identification of components was accomplished by relying on their retention indices, and the interpretation of the mass spectrum was carried out utilizing the database of the National Institute of Standards and Technology (NIST). This extensive database encompasses over 62,000 patterns of known compounds. To elucidate the composition of the unknown components within the obtained *Ficus racemosa* fraction, their spectra were systematically compared with the standard mass spectra of known components archived in the NIST library (NISTII). This comparative analysis facilitated

the accurate identification of the constituents present in the sample.

Results and Discussion

Phytochemical screening of *Ficus racemosa* methanolic fruit extract shows the presence of alkaloids, tannins, flavonoids, proteins and amino acids as shown in the following Table-2 & Fig.2.

Table 2: Phytoconstituents of *Ficus racemosa* fruit methanolic extract

Phytochemicals	Test	Presence(+) / Absence(-)
Flavonoids	Lead Acetate	+
	NaOH	+
Alkaloids	Dragendroff's	+
Proteins	Xanthoproteic	+
Aminoacids	Ninhydrin's	+

+ indicates the presence and - indicates the absence of phytochemicals in the plant extract



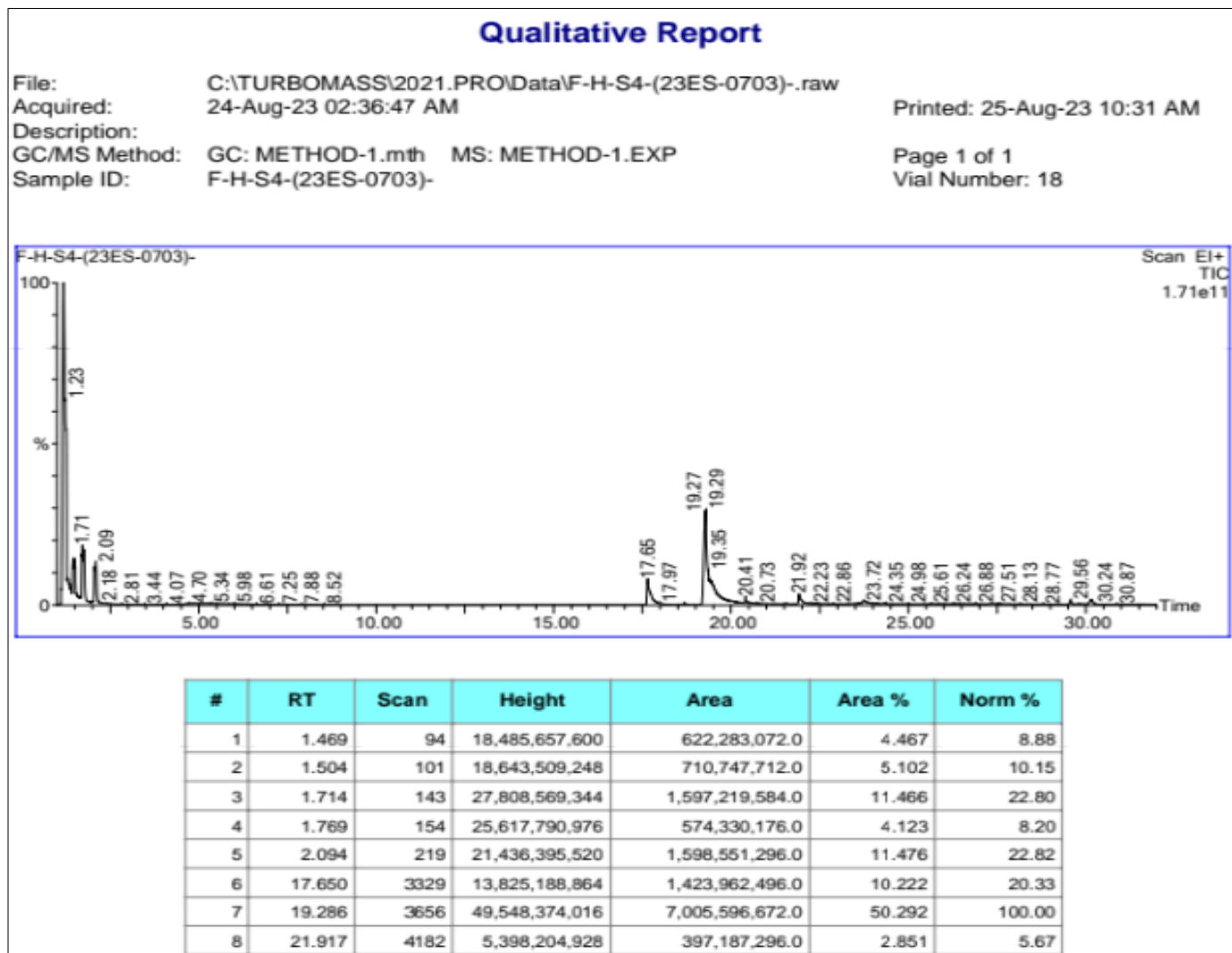
Fig 2: Phytochemical Screening of *Ficus racemosa* fruit methanolic extract

GC-MS analysis

Identification of compound

GC-MS is a unique analysis technique used for identification and quantification which is limited to analytes that are not only volatile and thermally labile but can also withstand the partitioning conditions of the gas chromatograph. A representative as shown in Fig.3 spectral output of all the certain able compounds from the empirical sample is displayed by this technique. The Gas chromatography device has an injection port from where the process is initiated by injecting the sample to that port. After this, evaporation and

separation of the components take place one by one and finally this equipment identifies the components present in the corresponding sample. A specific spectral pick is produced for each component which is recorded on a paper chart electronically. By using this technique we found some 8 chemical compounds in *Ficus racemosa* fruit methanolic extract, the compounds such as Cyclobutanethiol, 1,4-Oxathian-2-one, 6-methyl Thiazolidine, 2-methyl 4-Methoxy-1-butanol, 3Methylbutanoic acid, ethyl ester 4-Methyl-2,3-pentenediol, 2-Heptadecenal, 1,11-Dodecadiene based on the qualitative reports.

Fig 3: GC-MS chromatogram of *Ficus racemosa* fruit methanolic extractTable 3: GC- MS Analysis of *Ficus racemosa* fruit methanolic extract

S. No.	RT Value	Name of the compound	Molecular Formula	Molecular weight	Peak area	Structure of the Compound
1.	1.469	Cyclobutanethiol	C ₄ H ₈ S	88	4.467	
2.	1.504	1,4-Oxathian-2-one, 6-methyl	C ₅ H ₈ O ₂ S	132	5.102	
3.	1.714	Thiazolidine, 2-methyl	C ₄ H ₉ NS	103	11.466	
4.	1.769	1-Butanol, 4-methoxy	C ₅ H ₁₂ O ₂	104	4.123	
5.	2.094	Butanoic acid, 3-methyl, Ethyl ester	C ₆ H ₁₄ O ₂	130	11.476	
6.	17.65	(SR)or(RS)- 4-methyl- 2,3-pentenediol	C ₆ H ₁₄ O ₂	118	10.222	
7.	19.286	2-Heptadecenal	C ₁₇ H ₃₂ O	252	50.292	
8.	21.917	1,11-dodecadiene	C ₁₂ H ₂₂	166	2.851	

It's important to note that some of the compounds listed like Cyclobutanethiol, 1,4-Oxathian-2-one, 6-methyl, Thiazolidine, 2-methyl, and 1,11-Dodecadiene, are not commonly studied for their medicinal uses, and information about their specific medicinal properties may be limited or unavailable. However, there are some general insights into the potential medicinal uses of the compounds based on their chemical structures and properties:

Cyclo-butanethiol: Cyclobutanethiol is a cyclic thiol compound. Thiol compounds, including cyclobutanethiol, may have applications in organic synthesis and chemical reactions due to their reactivity and ability to form strong bonds with other molecules.

1, 4-Oxathian-2-one, 6-methyl: This compound belongs to the oxathiane class, which is a type of heterocyclic compound containing a sulfur atom. Oxathianes have been investigated in organic chemistry for their potential as building blocks in synthesis.

Thiazolidine, 2-methyl: Thiazolidines are another class of heterocyclic compounds containing sulfur and nitrogen atoms in the ring structure. Thiazolidines and their derivatives have been studied for various biological activities, including antimicrobial, antiviral, and anticancer properties.

4-Methoxy-1-butanol: Methoxy alcohols, like 4-methoxy-1-butanol, are organic compounds containing a methoxy (-OCH₃) group attached to an alcohol (hydroxyl) functional group. While methoxy alcohols have applications in organic synthesis and industrial processes.

3-Methylbutanoic acid, ethyl ester: This compound is an ester derivative of 3-methylbutanoic acid, which is a naturally occurring compound found in various foods and beverages. Esters are commonly used as flavoring agents and fragrances, but their medicinal uses are limited.

4-Methyl-2,3-pentanediol: This compound is a diol, which is a type of alcohol containing two hydroxyl (-OH) groups. Diols have applications in organic synthesis and as solvents, but specific medicinal uses for 4-methyl-2,3-pentanediol may not be extensively studied.

2-Heptadecenal: This compound is an aldehyde, which is a class of organic compounds containing a carbonyl group (-CHO). Aldehydes are found in nature and have applications in fragrance and flavor industries.

1, 11-Dodecadiene: Dodecadienes are hydrocarbons containing a double bond and eleven carbon atoms in the chain. While hydrocarbons have various industrial applications.

Conclusion

The field of pharmacology and phytochemistry, encompassing the study of herbal medicine, involves the screening of drugs in various ways, including assessment of physiochemical characteristics, mode of action, and therapeutic outcomes. The pharmacological investigation of herbal medicine is still in its nascent stages in many respects. Following the review, there is no doubt that the diverse medicinal herb *Ficus racemosa* is under scrutiny for a multitude of biological functions(52). This review

unequivocally highlights that *Ficus racemosa*, a versatile medicinal plant, is undergoing investigation for numerous biological activities. The phytochemistry and biological activity of various components of *Ficus racemosa* have been extensively researched over the past few decades. *Ficus racemosa* stands out as a rich source of diverse chemicals with varying chemical structures^[53]. Multidisciplinary research interventions have facilitated the transformation of underutilized fig fruit into valuable ingredients in the food sector. Despite this, the biological activity and potential medical uses of these substances have received limited research attention, warranting further study to harness their medicinal potential in combating diseases. Moreover, the availability of aqueous extracts has spurred scientists to delve deeper into investigating additional details concerning this medicinal plant to capitalize on its profit potential. Extensive research and development efforts should be directed toward *Ficus racemosa* due to its promising economic prospects for therapeutic applications^[54].

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